Soil Biology & Biochemistry 39 (2007) 2555–2566
Soil Biology & Biochemistry
www.elsevier.com/locate/soilbio

Does the enhanced P acquisition by maize following legumes in a rotation result from improved soil P availability?

Pieter Pypers[a,*], Michael Huybrighs[b], Jan Diels[a], Robert Abaidoo[c,1], Erik Smolders[b], Roel Merckx[b]

[a] *Tropical Soil Biology and Fertility Institute of the International Centre for Tropical Agriculture (TSBF-CIAT), P.O. Box 30677, Nairobi, Kenya*
[b] *Division Soil and Water Management, Department of Land Management and Economics, K.U.Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium*
[c] *International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320 Ibadan, Nigeria*

Received 7 December 2006; received in revised form 23 April 2007; accepted 27 April 2007
Available online 4 June 2007

Abstract

Field data have suggested that under P-deficient conditions, legumes supplied with phosphate rock (PR) increase P acquisition by a subsequent maize crop compared to direct application of PR to maize. This study assessed the mechanism of this positive rotational effect in terms of soil P availability using a greenhouse trial with large volume (74 l) containers. The rotation effect was analysed in relation to PR application, previous legume growth and incorporation of the legume residues. Velvet bean (*Mucuna pruriens*) and maize were grown in a representative Acrisol from the Nigerian Northern Guinea savannah (NGS). All soils were applied with sufficient urea to exclude N-effects in the rotations. In a first season, velvet bean and maize responded similarly to PR application, and P uptake by both crops increased by 45%. The soil total labile P quantity (*E*-value) and P concentration in soil solution ($^{31}P_{solution}$) after plant growth were increased by PR-application only in soils previously grown by velvet bean, suggesting enhanced PR solubilisation in the legume-grown soils. In the subsequent season, grain yields and P uptake of a maize crop following velvet bean were twice as large compared to maize following a first maize crop. This residual effect of velvet bean was even significant in treatments without PR-application, although both maize and velvet bean withdrew similar amounts of P during the first season and no differences in soil P availability were observed. Furthermore, legume residue incorporation in soils previously grown by maize did not affect yields or P uptake of the subsequent maize crop, while it significantly increased the *E*-value and $^{31}P_{solution}$ during the first 7 weeks in the second season. As such, the positive rotational effects of velvet bean were larger than predicted by soil P availability measures. Maize yield significantly increased with increasing plant P concentration among all treatments. However, the rotational effect was unrelated to internal P concentration: significantly larger yields were obtained for maize following velvet bean than for maize following maize at identical internal P. This suggested the presence of another growth-limiting which is counteracted by the previous velvet bean growth. In conclusion, our results confirmed that the introduction of a legume supplied with PR into a maize-based cropping system increases yield and P-uptake by a subsequent maize crop, compared to maize following a first maize crop supplied with PR. These stimulations, however, went beyond improved P nutrition. Results strongly suggested that the legume in the rotation system has other positive, possibly soil-microbiological effects which enhance maize growth and production.

Keywords: *E*-value; Legume–cereal rotation; Maize; *Mucuna pruriens*; P acquisition; P deficiency; P uptake; Phosphate rock; Soil solution P; Residue incorporation

1. Introduction

P deficiency limits crop production in many soils of the Northern Guinea savannah (NGS) zone of West-Africa (Bationo et al., 1998; Vanlauwe et al., 2002). Inorganic P fertilizers are often unavailable on local markets and/or not within economic reach of smallholder farmers, and

*Corresponding author. Tel.: +254 27224774; fax: +254 27224763.
E-mail address: p.pypers@cgiar.org (P. Pypers).
[1] Mailing address: IITA c/o Lambourn (UK) Limited, Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, UK.

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.04.026

therefore, alternative strategies are needed to improve P nutrition of crops. In the tropics, many phosphate rock (PR) deposits are mined but due to their low solubility, they are rarely used as a direct P input. Response to PR application by maize is usually limited in non-acid soils. Vanlauwe et al. (2000a, b), however, have shown that the utilization efficiency of PR can be improved by using adapted legume-maize rotation systems. Specific legumes are known for their ability to chemically alter P speciation in the rhizosphere and mobilize sparingly soluble P (Braum and Helmke, 1995; Kamh et al., 1999b; Pypers et al., 2006b). The main mechanisms resulting in enhanced utilization of PR are acidification of the rhizosphere (Bekele et al., 1983; Hinsinger and Gilkes, 1997) and exudation of organic acids (Hoffland, 1992). It is speculated that in this way, legumes revalue the PR into a more available P source. A cereal crop following the legume can then benefit directly from the enhanced P availability in the soil and acquire P released from the decomposing legume residues (Horst et al., 2001).

Velvet bean (*Mucuna pruriens*) has become increasingly popular with West-African farmers, mainly due to its weed-suppressing capacity (Tarawali et al., 1999). In addition, if well nodulated, velvet bean is able to biologically fix substantial amounts of atmospheric N_2 (Sanginga et al., 1996; Houngnandan et al., 2000) and is known to generally improve soil fertility (Carsky et al., 1998). Evidence was provided by Vanlauwe et al. (200a, b) that the incorporation of this legume in a maize-based rotation combined with PR application can largely improve utilization of PR within the system, in comparison with PR-applied in a sole maize cropping system. They reported a site-specific response by velvet bean to PR application. The PR application increased biomass production of velvet bean at one of the three sites (the 'plateau site') characterized by the lowest Olsen P content and higher P sorption capacity. At the beginning of the following growth season, the Olsen P content was significantly increased in fields where previously velvet bean was grown compared to fields where previously maize was grown. Yields of maize following the legume with PR application were increased from 1.0 to 2.7 t ha^{-1} and P uptake was more than three-fold larger compared to maize following the legume without PR application. These beneficial rotation effects were ascribed to the larger PR utilization by the legume and consequent improved P availability in the soil, since maize following the legume without PR application did not respond to urea addition. In PR-applied soils, however, both maize following velvet bean and maize following a first maize crop responded significantly to additional urea application, implying that the yield increase in the velvet bean–maize rotation is at least partly attributed to improved N nutrition of maize. Hence, it remains unclear to what extent a PR-supplied legume directly improves P availability for a following maize crop. This question, along with the need to understand the soil processes underlying the assumed improved P availability in legume–maize rotation systems, triggered the research presented in this paper.

The rotation effects of the legumes can be related to the root-induced processes leading to dissolution of PR and to P release from mineralizing legumes. In a pot trial, Kamh et al. (1999a) studied rotational effects of several leguminous cover crops in P-deficient soils of the Nigerian NGS. They showed that the growth of a maize plant following the legumes was principally P-limited. Growth and P uptake were enhanced for maize plants following legumes which showed high exudation rates of organic acids or phosphatases, examined independently in a hydroponics trial, which suggested that beneficial rotation effects were linked with the P mobilization capacity of the cover crop. Changes in P availability in the soil were however not studied. New methods based on isotopic dilution are now available to investigate changes in P speciation, bypassing some of the detection limits commonly encountered when measuring P concentrations in solution of weathered low-P soils (Maertens et al., 2004). In contrast with empirical P tests using chemical extraction, isotopic methods offer a mechanistic approach to study P. These methods consider the available P pool as the fraction of phosphate in the soil solution plus the exchangeable phosphate on the soil solid phase. The latter is termed the 'P quantity' while the phosphate concentration in soil solution is termed 'P intensity' (Dalal and Hallsworth, 1977). A third factor, the P buffering capacity, describes how the P quantity varies with a change in P intensity. It is proven that the labile P assessed using isotopic methods corresponds with the fraction of soil P accessed by plant roots (Russell et al., 1954; Fardeau, 1993; Frossard et al., 1994). We applied these enhanced methods to investigate the underlying mechanisms of improved P utilization in legume–cereal rotation systems.

The objectives of this research were: (i) to validate the beneficial effects of PR application in a legume–maize rotation system on P acquisition by a subsequent maize crop, in comparison with a sole maize cropping system under controlled conditions; (ii) to examine the effects of legume growth and legume residue incorporation on P speciation in a PR-applied soil; (iii) to link changes in P speciation in the soil with P uptake by the subsequent maize crop and verify whether improved P acquisition is truly a direct result of improved P availability.

2. Materials and methods

2.1. Setup of the container rotation trial in a greenhouse

Topsoil (0–15 cm) and subsoil (15–45 cm) were sampled from fallow land near Kasuwan Magani in the NGS of Nigeria. The soil was classified as a Ferric Acrisol with a petroferric phase (FAO, 1991), and is described in more detail by Vanlauwe et al. (2000a) where the soil is referred to as 'plateau field'. Topsoil consisted of the Ap horizon (0–9 cm) and the upper part of the Btc horizon (9–21 cm),

while the subsoil comprised the lower part of the Btc horizon and part of the Bt horizon (21–56 cm). The topsoil and subsoil were air-dried and passed through a 4 mm sieve. Selected physico-chemical properties of the top and subsoil are presented in Table 1. Sixty-four containers were installed in a greenhouse at IITA-Ibadan, Nigeria and filled with 42 kg of subsoil and 33 kg of topsoil. The containers were constructed by placing a black nylon bag, perforated at the bottom, in a square steel tube (40 cm high and 43 × 43 cm wide), mounted on a coarse gravel bed covered with a thin layer (about 1 cm) of river sand to ensure good drainage.

Table 1
Selected soil properties of the unamended top- and subsoil sampled near Kasuwan Magani (Nigeria)

		Topsoil (0–15 cm)	Subsoil (15–45 cm)
Sand[a]	(%)	47	37
Silt[a]	(%)	31	23
Clay[a]	(%)	22	40
pH (0.01 M $CaCl_2$)		4.69	4.84
Total C[b]	(%)	1.06	0.61
Total N[c]	(%)	0.085	0.051
CEC[d]	($cmol_c\,kg^{-1}$)	5.31	8.30
Ca^{2+}	($cmol_c\,kg^{-1}$)	1.12	3.26
Mg^{2+}	($cmol_c\,kg^{-1}$)	0.26	1.00
K^{+}	($cmol_c\,kg^{-1}$)	0.11	0.11
Exch. acidity[e]	($cmol_c\,kg^{-1}$)	0.17	0.17
Bray-I P[f]	($mg\,P\,kg^{-1}$)	8.43	0.69
E-value[g]	($mg\,P\,kg^{-1}$)	11.7	2.21
K_D[h]	($l\,kg^{-1}$)	2950	17 300
$^{31}P_{solution}$[i]	($mg\,P\,l^{-1}$)	0.0039	0.0001

[a]particle size analysis (IITA, 1982);
[b]organic carbon determined by wet combustion (Amato, 1983);
[c]Kjeldahl N (IITA, 1982);
[d]cation exchange capacity and exchangeable bases by ammonium-acetate method at pH 7.0 (IITA, 1982);
[e]exchangeable acidity determined by titration (IITA, 1982);
[f]Bray-I P (IITA, 1982);
[g]*E*-value determined by the method of Maertens et al. (2004) in a 1:10 0.01 M $CaCl_2$ extract after 48 h of isotopic exchange and 8 h of resin extraction;
[h]K_D determined in a 10:1 0.01 M $CaCl_2$ extract after 48 h of isotopic exchange;
[i]P concentration in the 0.01 M $CaCl_2$ extract calculated using $E\text{-value} = {}^{31}P_{solution}(K_D + L{:}S)$, with L:S = liquid solid ratio (Maertens et al., 2004).

A legume–maize rotation system was compared to a sole maize cropping system. We imposed 12 different treatments. The first eight treatments with six replications were established as a complete factorial design with three factors: first crop (legume or maize), PR application (with or without PR-applied to the first crop) and residue management (aboveground biomass of the first crop exported or incorporated). In another two treatments (T1 and T2) with four replications, legume residues were imported into the sole maize cropping system, with or without PR application to the legume. The remaining two treatments (R1 and R2) with four replications were reference legume–maize systems, with or without PR-applied to the legume and TSP applied in the second season to the maize. Residues of the first crop were exported in both the R1 and R2 treatment. The treatment structure is presented in Table 2. The placement of the containers with the various treatments in the greenhouse was completely randomized.

Sieved (0.15 mm) PR from Hahotoe, Togo (total P content = 11.1% P; soluble P in 2% citric acid = 3.3% of PR) was applied at a rate of 15.0 g PR per container, by spreading the PR over the soil surface and raking it into the top 10 cm dry topsoil. This corresponds to a field rate of 90 kg P ha^{-1}, assuming a maize spacing of 15 cm within row and 75 cm between rows (53,333 maize plants per hectare). A nutrient solution containing $Mg(NO_3)_2$, KCl, $CaCl_2$, $MgSO_4$, $ZnCl_2$, $CuCl_2$, $FeCl_3$, $MnCl_2$, $CoCl_2$, $(NH_4)_6Mo_7O_{24}$, and $Na_2B_4O_7$ salts was poured over the soil surface of every container. This blanket nutrient application provided a starter dose of N (20 kg N ha^{-1}) and sufficient amounts of other macro- (except P) and

Table 2
Treatment structure for comparing a legume–maize rotation system and a sole maize cropping system as affected by PR application and residue management

Treatment	First crop, supplied with		Second crop, supplied with	
Maize(0P)/maize(−r)	Maize	—	Maize	—
Maize(0P)/maize(+r)	Maize	—	Maize	Residues of 1st crop
Maize(90PR)/maize(−r)	Maize	90 kg PR-P ha^{-1}	Maize	—
Maize(90PR)/maize(+r)	Maize	90 kg PR-P ha^{-1}	Maize	Residues of 1st crop
Velvetbean(0P)/maize(−r)	Velvet bean	—	Maize	—
Velvetbean(0P)/maize(+r)	Velvet bean	—	Maize	Residues of 1st crop
Velvetbean(90PR)/maize(−r)	Velvet bean	90 kg PR-P ha^{-1}	Maize	—
Velvetbean(90PR)/maize(+r)	Velvet bean	90 kg PR-P ha^{-1}	Maize	Residues of 1st crop
T1: maize(0P)/maize(+rVelB)	Maize	—	Maize	Residues of velvet bean, grown with PR supply
T2: maize(90PR)/maize(+rVelB)	Maize	90 kg PR-P ha^{-1}	Maize	Residues of velvet bean, grown without P supply
R1: velvetbean(0P)/maize(+60TSP)	Velvet bean	—	Maize	60 kg TSP-P ha^{-1} Residues of 1st crop exported
R2: velvetbean(90PR)/maize(+60TSP)	Velvet bean	90 kg PR-P ha^{-1}	Maize	60 kg TSP-P ha^{-1} Residues of 1st crop exported

micronutrients (100 kg K ha^{-1}, 60 kg Ca ha^{-1}, 40 kg Mg ha^{-1}, 17 kg S ha^{-1}, 0.6 kg Fe ha^{-1}, 0.6 kg Mn ha^{-1}, 0.3 kg Zn ha^{-1}, 0.3 kg Cu ha^{-1}, 0.15 kg Co ha^{-1}, 0.15 kg Mo ha^{-1}, and 0.15 kg B ha^{-1}). Only to containers where maize was grown as first crop, additional urea N was split-applied during plant growth at a total rate equivalent to 160 kg N ha^{-1} (15%, 20%, 30% and 35% of the total rate at 10, 20, 32 and 45 days after planting (DAP), respectively). The urea was added in a small furrow (20 cm long and 3 cm deep) about 10 cm from the maize base and covered.

Per treatment, two 20 cm TDR (time domain reflectometry) probes were installed vertically in the soil, and the volumetric moisture content was determined daily during the trial. Based on these moisture contents, water loss from the containers was calculated. The first five days, the moisture content was increased by daily adding 2 l of distilled water to every container. The moisture content was then increased more gradually to 20% v/v, and corrected daily during crop growth based on the TDR measurements.

Velvet bean (*Mucuna pruriens* (L.) D.C. cv. utilis (Wright) Burck) and maize (*Zea mays* L. cv. Oba Super II) were selected as test crops. The maize cultivar is a commercial hybrid, late-maturing variety and is known to be N-efficient (Sanginga et al., 2003). Velvet bean seeds were pre-germinated in moist cotton wool. Four days after water addition had started, five maize or velvet bean seeds were planted in each container. Seven DAP, the seedlings were thinned to one maize plant or two velvet bean plants per container. During crop growth, the containers were weeded and occurrence of pests and diseases was controlled by regular preventive spraying. Seedlings (7 DAP) were sprayed with a fungicide (Benlate®, 2 g l^{-1}, active compound = benomyl), and an insecticide mixture (Sherpa plus®, 2 ml l^{-1}, active compounds = cypermethrin and dimethoate). Insecticide spraying was repeated 3, 6 and 10 weeks after planting. In the containers with velvet bean, rods (2.5 m) were installed to allow the legume to climb. Growing across neighbouring plants was avoided by regularly returning the offshoots of velvet bean to the rod.

The velvet bean aboveground biomass was harvested earlier than the maize. Pod formation had started and in order to obtain high quality residues without translocation of nutrients to the seeds, the velvet bean aboveground biomass was cut 98 DAP. The maize was harvested 105 DAP. The cobs were separated from the shoots and plant materials were dried in an oven at 70 °C. Dried maize and velvet bean plant shoots were cut to pieces not larger than 2 cm and a subsample of 10 g cut residues was ground. Ground plant samples were digested in concentrated hot H_2SO_4 and the N and P contents were determined colorimetrically using automated analysis (TECHNICON™ AUTOANALYZER™ II System) by the methods of Novozamsky et al. (1983) and Murphy and Riley (1962).

After the first crop was harvested, the soil in the containers was allowed to dry for 10 days. The rods and maize stubbles were removed and the topsoil was re-homogenized. Five days after rewetting, the total amount of residues from the preceding crop was incorporated into the top 10 cm soil for the relevant treatments. In the reference treatments with TSP application, TSP was applied at a rate equivalent to 60 kg P ha^{-1}. At the start of the second season, macro- and micronutrients were added similarly and at the same rates as in the first season to all containers. As some indications of potassium deficiency stress had been observed in the first season, the application of K and other macronutrients (Ca, Mg and S) was repeated after 33 days in the second season at the same initial rates but only in treatments where no velvet bean residues were incorporated. Velvet bean residues contained about 0.8% K, representing an application rate equivalent to 160 kg K ha^{-1}; this K is released readily, during the initial 4 weeks of decomposition (Cobo et al., 2002) and is thus almost directly available for plant uptake. In treatments without velvet bean residue application, urea application was also doubled, compared to the first season. No urea was applied in treatments with incorporated velvet bean residues. The velvet bean residues obtained contained about 2.1% N and represented an N input equivalent to 430 kg N ha^{-1}. Ibewiro et al. (2000) showed that decomposing velvet bean residues readily release N which can then be utilized by a maize crop. Assuming that half of the N in the residues is released during the second season, these residues can amply supply N to the subsequent maize crop. Planting, harvesting (at 107 DAP) and analysis of plant materials in the second season was done similarly to the first season.

2.2. Soil sampling and analysis

During plant growth, composite topsoil samples (three samples per container, at least 10 cm away from the maize or velvet bean base) were taken using an auger with a plastic liner (inner diameter 2.35 cm, depth 7.5 cm). In every treatment, four replicate containers were sampled. After sampling, the holes were filled with control topsoil and a marker was placed to avoid the spot at consecutive sampling times. Samples were taken 37 and 104 DAP during the first season, and 2, 16, 29, 48, 75 and 99 DAP during the second season. After recording the total fresh weight, the samples were passed through a 2 mm sieve and roots and residues were removed. Part of the sample was dried and the moisture content was determined. The remaining fresh sample was stored in a fridge (5 °C).

Soils were analysed for P availability in terms of P quantity (Q is the total amount of labile P in the soil), P intensity (I is the phosphate activity in the soil solution) and P buffering capacity ($PBC = \delta Q/\delta I$). The labile P quantity was estimated by the E-value, using the method of Maertens et al. (2004), and was determined on dried soil samples, as drying is unlikely to affect the E-value. An aliquot of 3 g soil was equilibrated with 29 ml water by shaking end-over-end during 16 h. Consecutively, 1 ml of a

carrier-free ^{32}P-orthophoshate solution (65 kBq ml^{-1}) was added and samples were remounted on the shaker. After 48 h, 1 strip of an anion exchange membrane (AEM, 6 × 1 cm, product 55164 2S; BDH Laboratory supplies, Poole, England), previously converted to the bicarbonate form, was added and shaken with the soil suspension for another 8 h. The resin strips were washed, transferred to 20 ml of 0.5 M HCl and shaken during 16 h. The P concentration in the resin extract was determined by the malachite green method and the ^{32}P activity in the resin extract was determined by LSC in a Packard Tri-Carb 1600CA liquid scintillation system. The *E*-value was calculated using Eq. (1):

$$E = D/\mathrm{SA_{AEM}} = D/(^{32}\mathrm{P_{AEM}}/^{31}\mathrm{P_{AEM}}) \quad (1)$$

with E = the *E*-value [mg P kg^{-1}], D = the applied ^{32}P dose [Bq kg^{-1}], $\mathrm{SA_{AEM}}$ = the specific activity in the AEM extract [Bq (mg P)$^{-1}$], $^{32}\mathrm{P_{AEM}}$ = the ^{32}P activity in the AEM extract [Bq l^{-1}] and $^{31}\mathrm{P_{AEM}}$ = the P concentration in the AEM extract [mg P l^{-1}].

The ^{32}P distribution coefficient (K_D), an estimator for the P buffering capacity (PBC), and the P concentration in soil solution ($^{31}\mathrm{P_{solution}}$), an estimator for the P intensity (*I*), were measured on fresh soil samples, since these parameters are highly susceptible to presence of labile organic compounds. To determine the ^{32}P distribution coefficient (K_D, [l kg^{-1}]), an aliquot of soil equivalent to 3 g dry soil was weighed into polyethylene centrifuge tubes and 29 ml of 0.01 M $CaCl_2$ was added. The soil suspension was equilibrated on a vertical shaker during 16 h. Consecutively, 1 ml of a carrier-free ^{32}P-orthophosphate solution (100 kBq ml^{-1}) was added and samples were remounted on the shaker. After 48 h, the soil suspensions were centrifuged (20 min at 9000 *g*) and filtered (MF-Millipore, 0.22 μm). To 1 ml of filtrate, 4 ml of LS cocktail ULTIMA GOLD XR was added and samples were counted in a Beckman LS5000CE liquid scintillation system. The K_D-value was calculated as the ratio of ^{32}P activity adsorbed over ^{32}P activity remaining in solution. Since velvet bean residue application affects the soil pH, and thus the solubility of PR, the influence of pH on the K_D-value was studied in treatments with maize following velvet bean (except R1 and R2). In these treatments, the K_D was determined on dried soil samples but prior to the initial 16 h equilibration period, the pH was adjusted using 1 M HNO_3or 1 MNaOH to obtain pH values within a range of 3.5–7.5.

The P concentration in soil solution ($^{31}\mathrm{P_{solution}}$, mg P l^{-1}) was only directly assessed in soils applied with TSP. An aliquot equivalent to 3 g dry soil was equilibrated with 30 ml 0.01 M $CaCl_2$ during 16 h on a vertical shaker. Subsequently, the suspension was centrifuged, filtered and the P concentration in solution was determined by the malachite green method (Van Veldhoven and Mannaerts, 1987). In all other soils, P concentrations in solution were generally below the detection limit of the malachite green method (8 μg P l^{-1}) and were calculated based on the *E*- and K_D-value (Maertens et al., 2004):

$$^{31}\mathrm{P_{solution}} = E/(K_D + \mathrm{L{:}S}) \quad (2)$$

with E = the *E*-value [mg P kg^{-1}], K_D = the ^{32}P distribution coefficient [l kg^{-1}] and L:S = the liquid solid ratio [l kg^{-1}].

2.3. Statistical analyses

The SAS program (SAS, 1999) was used to perform analysis of variance (ANOVA). Significance of effects of crop species, previous crop, PR application and residue incorporation on yield, P uptake, soil pH and P availability measures was determined using a general linear model (GLM procedure) and a least square means method to calculate linear combinations of the estimated effects; least significant differences at $P<0.05$ ($\mathrm{LSD_{0.05}}$) were used for comparing treatment effects. Relevant comparisons with additional treatments (T1, T2, R1 and R2, see Table 2) were conducted by computing estimates of differences between treatments using the ESTIMATE statement. Differences in the relation in P uptake and measures of P availability between selected treatments were tested by an analysis of covariance.

3. Results

3.1. Response by maize and velvet bean to PR application (first season)

The labile P quantity (*E*-value = 12 mg P kg^{-1}) and a very small P concentration in the soil solution (2 μg P l^{-1}) suggest that the soil is P-deficient. However, maize and velvet bean performed rather well in this trial (Fig. 1), which is likely due to the optimal growing conditions in the greenhouse. The maize yielded 125 g grains per plant, which in the field would correspond to a grain yield of 6.6 t ha^{-1}. Application of PR increased yields of both crops. Velvet bean supplied with PR yielded over 400 g aboveground dry matter per container, which is a considerable amount of residues to be incorporated, equivalent to a field rate of 22 t DM ha^{-1}.

P uptake was similar for maize and velvet bean. In the control, about 320 mg P per container was taken up, which is quite large considering that the control *E*-value in the topsoil equals 12 mg P kg^{-1} and the topsoil thus contains a total amount of only 386 mg labile P (after 48 h of isotopic exchange) per container. For both crops, PR application significantly increased P uptake by, on average, 145 mg P per container. As such, maize and velvet bean are equally able to utilize PR (about 9% of the rock phosphate applied) under these controlled conditions.

Even though both crops withdrew similar amounts of P from the soil, we observed differences in soil P availability indices in the PR amended soils between the two crops (Fig. 2). In the control treatments, the *E*-value decreased by

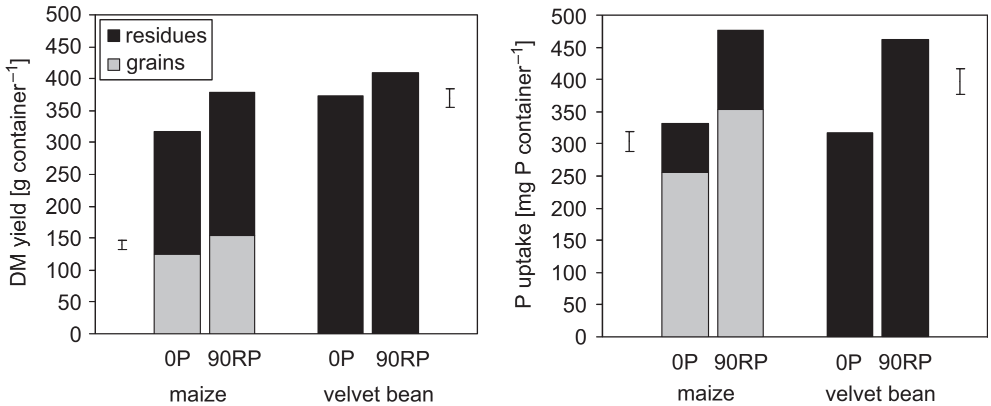

Fig. 1. Biomass, grain yields and P uptake of maize and velvet bean as affected by PR application (first season). Lower left error bars represent $LSD_{0.05}$ for comparison of maize grain yields and total P contents of grains; upper right error bars represent $LSD_{0.05}$ for comparison of total DM yield (residues + grains) and total plant P uptake of maize and velvet bean ($n = 16$).

almost 50% and $^{31}P_{solution}$ decreased from 2 to 0.7 μg P l^{-1} during the first season. In PR-applied soil grown with maize, the *E*-value did not change with time, while the *E*-value slightly increased with time in soils grown with velvet bean. The $^{31}P_{solution}$ remained significantly largest in containers where PR was applied and velvet bean was grown. In this treatment, the K_D did not change with time, contrary to the corresponding treatment where maize was grown, and where an increase in K_D and consequently, a decrease in $^{31}P_{solution}$ were observed at the end of the season.

3.2. *Response by maize to previous velvet bean growth, PR application and incorporation of legume residues (second season)*

Compared to the first season, control maize yields in the second season had significantly declined to 30 g grains per plant, equivalent to about 1.6 t ha^{-1} (Fig. 3). The DM and grain yields of maize following velvet bean were more than twice as large compared to maize following a first maize crop. This positive effect of previous velvet bean growth on maize yield and P uptake was highly significant (F-values = 28.2 and 86.6, $P<0.0001$) and occurred irrespective of whether PR was applied, or whether residues of the preceding crop were incorporated. The residual effect of velvet bean was even considerable in the treatments where the residues were removed, although both maize and velvet bean withdrew similar amounts of P during the first season. PR application significantly increased yields (F-values = 14.3, $P = 0.005$) and P uptake (F-values = 73.6, $P<0.0001$) of both maize following velvet bean and maize following maize. In the second season, maize following velvet bean utilized 11% of the applied rock phosphate versus 8% by maize following a first maize crop (calculated based on the difference with the control treatments without residue incorporation). The incorporation of legumes in maize-based cropping systems, combined with PR application increased yields of the subsequent maize crop compared to a maize monocropping system with PR application, achieving 72% of the maximal yield observed in the reference treatment with TSP application to maize (on average 160 g grains per plant, equivalent to 8.5 t ha^{-1}, treatments R1 and R2).

Residue management, however, did not affect maize grain yields in the second season. A significant (F-values = 4.0, $P = 0.05$) interaction between effects of the previous crop and effects of residue incorporation on total maize DM yield was observed. Incorporation of maize residues (containing on average 0.05% P) did not affect yields. Contrarily, the incorporation of velvet bean residues significantly increased total DM yields. Although P concentrations in the velvet bean residues were rather small (0.09% P in the control and 0.12% P in the treatment with PR), incorporating these residues nevertheless provided a substantial P input (equivalent to field rates of 17–25 kg P ha^{-1}) because of the high DM application rates. Grain yields, however, were not affected by residue application. In the additional treatments (T1 and T2), where velvet bean residues were imported into the sole maize cropping system, maize yields and P uptake were slightly larger than the control but smaller compared to yields obtained in soils where previously velvet bean was grown.

3.3. *Relation between plant P uptake and measures of P availability*

Both *E*-values and $^{31}P_{solution}$ were small during the entire second season in control soils irrespective whether previously maize or velvet bean was grown. As such, the larger P uptake by maize following velvet bean compared to maize following a first maize crop cannot be explained by a difference in the soil labile P quantity or P intensity. In PR-applied soils, *E*-values and particularly $^{31}P_{solution}$ remained consistently larger during the first 7 weeks in the second season if previously velvet bean was grown, compared to, if previously maize was grown. The improved soil P

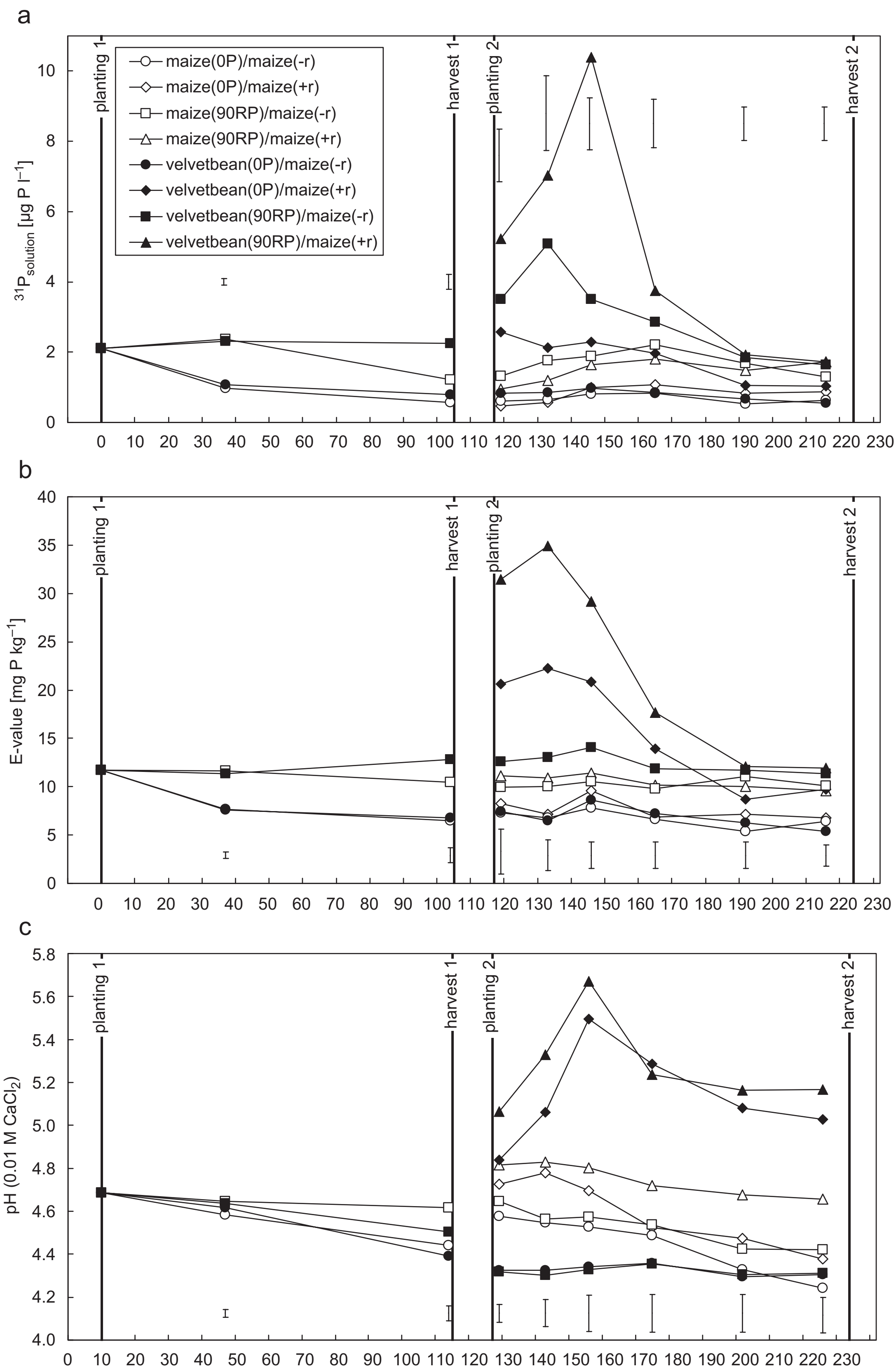

Fig. 2. Changes in topsoil (a) P intensity ($^{31}P_{solution}$), (b) labile P quantity (*E*-value) and (c) pH with time during both seasons as affected by the first crop (maize or velvet bean), PR application and residue management. Error bars represent $LSD_{0.05}$ at a given sampling time ($n = 4$).

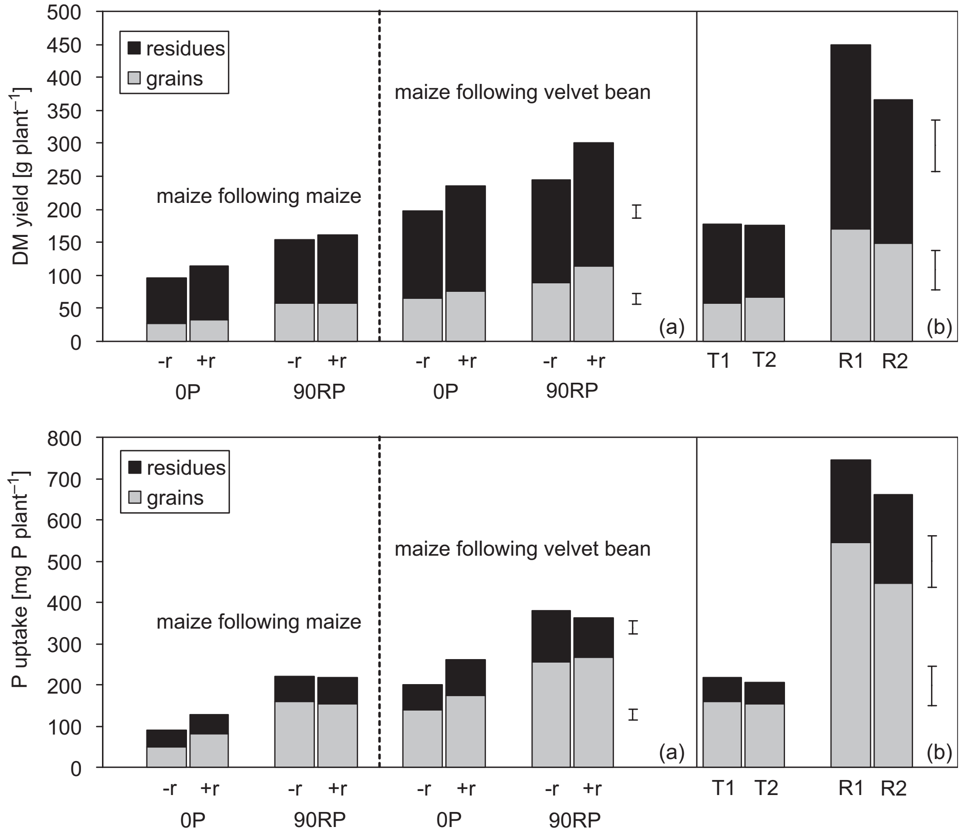

Fig. 3. Total residue and grain yields and P uptake of maize (second season) as affected by the previous crop (maize or velvet bean), PR applied to the previous crop and residue management. Additional treatments (see Table 2) include two treatments with application of velvet bean residues to maize following a first maize crop (T1 and T2) and reference treatments with TSP application (R1 and R2). Lower error bars represent $LSD_{0.05}$ for comparison of maize grain yields and total P contents of grains; upper error bars represent $LSD_{0.05}$ for comparison of total DM yield (residues + grains) and total plant P uptake ($n = 6$); error bars marked (a) represent $LSD_{0.05}$ for first eight treatments, error bars marked (b) represent $LSD_{0.05}$ for all 12 treatments.

availability demonstrates that growing a legume enhances P release from the PR-applied. However, incorporation of velvet bean residues almost tripled *E*-values and $^{31}P_{solution}$ between 16 and 29 DAP while no significant effects of residue incorporation on maize grain yields were observed. In the additional treatments T1 and T2, incorporation of velvet bean residues also almost tripled *E*-values between 16 and 29 DAP. The $^{31}P_{solution}$ differed significantly among treatments, with largest P concentrations observed in the treatment where residues of velvet bean previously grown with PR application were incorporated in control soil (T1), compared to the treatment with incorporation of control velvet bean residues in soil where previously PR was applied (T2). Maximal $^{31}P_{solution}$ equalled 6 and $3\,\mu g\,P\,l^{-1}$ in the treatments T1 and T2, respectively. Although incorporation of velvet bean residues led to large increases in P quantity and P intensity, effects on plant P uptake were very limited. As such, beneficial effects of the legume on P uptake by the subsequent maize crop proved to be larger than predicted by the P availability measures.

This is confirmed by a correlation analysis, relating total P uptake by maize in the second season to measures of soil P availability determined 29 DAP (third sampling time in the second season); at this sampling time, maximal treatment effects were observed and the maize plants had well advanced into their exponential growth phase. We found significant but rather weak correlations between total P uptake by maize and $^{31}P_{solution}$ or the *E*-value, excluding treatments with addition of TSP (Table 3). Measures of P availability can but partly explain the observed positive rotational effects. Correlations were improved by including the K_D-value and the pH in a multiple regression equation with $^{31}P_{solution}$ $R^2 = 0.76$). The effect of changes in soil pH on the K_D-value was investigated in more detail for selected treatments (Fig. 4). Without PR application, a similar plateau K_D-value (about $3650\,l\,kg^{-1}$) was observed between pH 4.5 and 6.0 in the control and in the soil amended with velvet bean residues. In PR-applied soil, a slightly larger plateau K_D-value ($4070\,l\,kg^{-1}$) was observed at pH 4.5–6.0 if residues were exported. Incorporation of velvet bean residues generally decreased the K_D-value over the entire pH range. Through mineralization, P and organic anions were released and sorption sites in the soil were presumably occupied. In both treatments in PR-applied soil, a pH decrease below pH 4.0 resulted in a considerable decrease

Table 3
Pearson correlation coefficients (R) and multiple regression equations relating total P uptake by maize in the 2nd season to measures of soil P availability ($^{31}P_{solution}$, *E*-value, K_D and soil pH ($n = 40$))

Variable(s)	R	Multiple regression equation
$^{31}P_{solution}$	0.75	P uptake = $794^{***} + 92.5^{***}\ln(^{31}P_{solution})$
$^{31}P_{solution} + K_D$	0.76	P uptake = $1220^{***} + 53.9^{*}\ln(^{31}P_{solution}) - 75.3^{ns}\ln(K_D)$
$^{31}P_{solution} + K_D + pH$	0.87	P uptake = $847^{***} + 299^{***}\ln(^{31}P_{solution}) + 241^{**}\ln(K_D) - 181^{***}pH$
E-value	0.54	P uptake = $108^{***} + 7.59^{***}$ *E*-value
E-value + K_D	0.75	P uptake = $1360^{***} + 2.72^{ns}$*E*-value $- 134^{***}\ln(K_D)$
E-value + K_D + pH	0.83	P uptake = $1330^{***} + 17.4^{***}$*E*-value $- 61.7^{*}\ln(K_D) - 167^{***}pH$

^{ns}, *, ** and *** indicate a non-significant contribution in the equation of the coefficient at $P<0.05$ and significant contributions of the coefficient at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

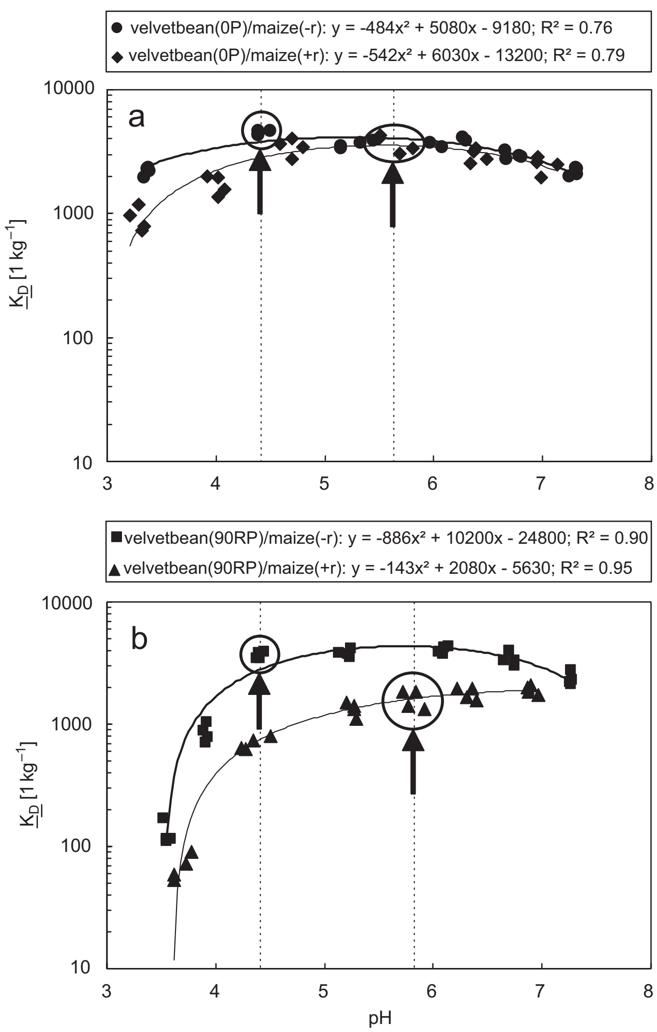

Fig. 4. K_D-value (29 DAP, second season) as affected by an experimental change in pH for selected soils grown with velvet bean in the first season, in treatments (a) without PR application or (b) with PR application, and with the residues either exported or incorporated at the beginning of the second season. Arrows mark the original pH of the soils.

in K_D and thus an increase in $^{31}P_{solution}$. At a pH of 3.6, $^{31}P_{solution}$ increased to 0.66 and 1.4 mg P l^{-1} in the treatment with residues exported and incorporated, respectively. As such, if velvet bean residues were exported, a pH decrease by 0.6 units in PR-applied soil resulted in an increase in $^{31}P_{solution}$ by almost a factor 200, while if residues were incorporated, a much larger pH decrease was required to increase $^{31}P_{solution}$. The soil pH thus has a considerable effect on P availability in PR-applied soils.

4. Discussion

Field results by Vanlauwe et al. (2000a) also showed increased P availability (in terms of Olsen P contents) in PR-applied soils after legume growth versus maize growth. This improved soil P availability is likely related to P mobilization mechanisms of velvet bean. Several authors have shown that legumes are able to access sparingly soluble P through root-induced chemical changes (e.g. Braum and Helmke, 1995; Kamh et al., 1999b; Hinsinger, 2001). The legumes decreased soil pH to a larger extent than maize (Fig. 2c). Acidifying the rhizosphere enables species to utilize PR. Hoffland (1992) demonstrated that exudation of organic acids was highly effective to increase P uptake from PR. Citrate release can significantly dissolve Ca-bound P (Jones and Darrah, 1994). In contrast to legumes, root-induced P mobilization is unlikely to occur in the rhizosphere of the maize variety studied (Pypers et al., 2006b). Nevertheless, maize and velvet bean responded comparably to PR application in the first season of our trial. Cereal crops importantly rely on morphological adaptations to efficiently utilize soil P resources (Gahoonia and Nielsen, 2004). Gahoonia et al. (1999) showed that the root hair length determined P uptake efficiency in barley cultivars grown in low-P soils. We suspect that the maize variety studied is capable of acquiring P at low P intensity due to its long root hairs (on average 0.68 mm; unpublished results from other trials). The optimal moisture conditions may have favoured the development of an extensive active root system which enabled maize to take up a relatively large amount of P (considering the *E*-value).

Our results confirm that supplying a legume with PR improves P availability in the soil and P acquisition by a subsequent maize crop, in comparison with a sole maize cropping system. However, we observed large discrepancies between measures of P availability and maize P uptake: correlation analysis revealed that measures of P quantity or P intensity can only account for 29% and 56 % of the total

variation in P uptake. Including the K_D-value improved the correlation, as P uptake is known to be affected by both the P quantity and the P intensity (Holford and Mattingly, 1976; Pypers et al., 2006a). Furthermore, the regression equation indicates that P uptake increases with decreasing pH, which is likely related to a better availability of the applied PR in treatments with lower pH. Particularly the incorporation of velvet bean residues led to large increase in soil pH. We conducted an analysis of covariance to investigate the relation between maize P uptake and $^{31}P_{solution}$ as affected by the incorporation of velvet bean residues in PR-applied soils. In treatments where velvet bean residues were incorporated (pH 5.7–5.9), maize required a significantly larger ($P<0.01$) $^{31}P_{solution}$ to obtain a given P uptake, compared to other treatments where the pH remained low (pH $= 4.3-4.8$). Besides, incorporation of velvet bean residues significantly increased soil P availability, as shown by the E-value and $^{31}P_{solution}$ (Fig. 2). In the treatments with no incorporation of velvet bean residues, a slight further acidification in the rhizosphere possibly resulted in a substantial dissolution of the applied PR, as was illustrated in Fig. 4. We suspect that in treatments with combined PR and velvet bean residue application, the P taken up by the maize plant is mainly supplied by the decomposing residues. In PR-applied soils where residues were exported and the pH remained low, however, P is more likely supplied by the dissolution of the PR. Further studies with labelled residues and soils are required to confirm this hypothesis. Oladeji et al. (2006) also reported large pH increases when incubating a less acid soil (pH = 5.3) with plant residues of various qualities; combined plant residue and PR application, however, did not result in increased P release from PR in this soil. Other authors did observe that the combined application of PR and organic residues leads to improvements in P availability and crop yields (e.g.Sharma and Prasad, 2003; Waigwa et al., 2003; Tian and Kolawole, 2004). The effectiveness of the materials however depended largely on the soil and residue characteristics because P release from PR and residues is determined by complex interactions between the soil and the organic materials.

Since no reference treatment with TSP application was included in the sole maize system, it is difficult to verify whether P deficiency was the only limitation for crop growth. To examine to which extent the beneficial rotation effects on P uptake by the subsequent maize crop were attributed to improved P nutrition, yields were related to the internal P concentration (Fig. 5). Maize yield significantly increased with increasing plant P concentration among all treatments. However, the relationship between internal P concentration and DM yield varied with the previously grown crop. The DM yield of maize following a first maize crop remained largely unaffected by the internal P concentration; hence, maize accumulated P without a significant response in growth. For maize following velvet bean, larger DM yields were observed at a given internal P concentration compared to maize following maize, and

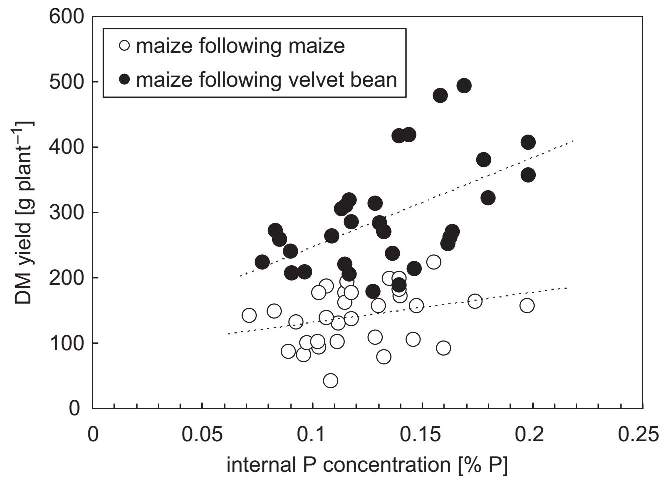

Fig. 5. Relationship between the maize DM yield (second season) and the internal P concentration, calculated as the ratio of the total P uptake over the DM yield, separate for the sole maize system and the velvet bean-maize rotation system, and combining data from all 6 treatments within each system ($n = 32$). Covariance analysis revealed a significant difference ($P<0.05$) between both systems.

DM yields significantly increased with increasing internal P concentration. This difference in internal P utilization strongly suggests the presence of another growth-limiting factor, other than P, which was counteracted by the legume growth. This factor is possibly microbiological in nature, related to changes in populations of arbuscular mycorrhizal fungi (Fyson and Oaks, 1990; Munkvold et al., 2004; Jansa et al., 2005), P-solubilizing fungi and bacteria (Whitelaw, 2000; Barea et al., 2002) or microorganisms with antagonistic properties towards plant microbial pathogens and nematodes (Vargas-Ayala et al., 2000), with possible beneficial interactions between these microbial species (Barea et al., 2005). Babana and Antoun (2006) showed that combined inoculation with PR-solubilizing organisms and a commercial arbuscular mycorrhizal fungi isolate not only increased P acquisition by wheat in a PR-applied soil, but also favoured improved internal P utilization. This suggests that shifts in mycorrhizal communities could likely be involved in the positive rotational effects observed in our trial. Our study was, however, not designed to investigate the possible contribution of soil microorganisms; it needs to be acknowledged that the biocides applied may have influenced soil microbial communities, and therefore obscured some of the positive rotational effects observed. Alvey et al. (2001) and Bagayoko et al. (2000) studied cereal–legume rotation systems in West-Africa and concluded that yield improvements in the rotation system versus continuous cereal growth were due to a combination of improved N and P nutrition, higher levels of arbuscular mycorrhiza and lower infection rates by plant parasitic nematodes. We concur with the above authors that future studies should focus on the biological fertility of legume–cereal rotation systems, with specific focus on plant-beneficial interactions between microorganisms occurring in the rhizosphere.

In conclusion, our results confirm that PR application, combined with the incorporation of velvet bean in a rotation system with maize, can considerably enhance crop production. The legume growth enhances P acquisition by the subsequent maize crop, but evidence was found that the benefits of the system go beyond N and P fertility. The soil pH as affected by crop growth and residue management crucially determines P availability and uptake in PR-applied soils. In addition, results suggested that the rotation system may eliminate other growth-limiting factors, likely microbiological in nature.

It needs to be stressed that incorporating a legume into the system cannot eliminate the need for P inputs. Because most PR deposits are mined in the tropics, one often considers PR as a suitable P source for crop production in tropical P-deficient soils (Sanchez et al., 1997). However, PR enjoys little popularity towards extension agencies and farmers. High rainfall and low soil pH are absolute prerequisites for PR utilization. Beneficial effects of PR application combined with adapted legume-cereal rotation systems are site-specific (Vanlauwe et al., 2000b). Also, management is difficult, PR is rarely locally available on markets, and has higher transportation costs than TSP because of its lower P content and solubility. In the end, cost-effectiveness determines whether farmers will apply PR (Buerkert et al., 2001). The profitability of a rotation system with velvet bean and PR application needs to be compared with direct application of soluble P fertilizer. Finally, rather than using a herbaceous legume such as velvet bean, the use of a dual-purpose grain legume (e.g. soybean), providing edible beans and a direct profit to farmers, could considerably accelerate the system's implementation and therefore deserves further investigation.

Acknowledgements

This work was funded by the Belgian Directorate General of Development Cooperation, as part of the collaborative project between K.U.Leuven and IITA on 'Achieving Development Impact and Environmental Enhancement through Adoption of Balanced Nutrient Management Systems by Farmers in the West African Savanna'. Pieter Pypers acknowledges a Ph.D. fellowship from the 'Fonds voor Wetenschappelijk Onderzoek Vlaanderen'. Michael Huybrighs acknowledges a grant from the VLIR-Interuniversity Collaboration Section.

References

Alvey, S., Bagayoko, M., Neumann, G., Buerkert, A., 2001. Cereal/legume rotations affect chemical properties and biological activities in two West African soils. Plant and Soil 231, 45–54.

Amato, M., 1983. Determination of ^{12}C and ^{14}C in plant and soil. Soil Biology & Biochemistry 15, 611–612.

Babana, A.H., Antoun, H., 2006. Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali. Plant and Soil 287, 51–58.

Bagayoko, M., Buerkert, A., Lung, G., Bationo, A., Romheld, V., 2000. Cereal/legume rotation effects on cereal growth in Sudanu-Sahelian West Africa: soil mineral nitrogen, mycorrhiza and nematodes. Plant and Soil 218, 103–116.

Barea, J.-M., Pozo, M.J., Azcon, R., Azcon-Aguilar, C., 2005. Microbial co-operation in the rhizosphere. Journal of Experimental Botany 56, 1761–1778.

Barea, J.-M., Toro, M., Orozco, M.O., Campos, E., Azcon, R., 2002. The application of isotopic ^{32}P and ^{15}N-dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. Nutrient Cycling in Agroecosystems 63, 35–42.

Bationo, A., Lompo, F., Koala, S., 1998. Research on nutrient flows and balances in West Africa: state-of-the art. Agriculture, Ecosystems and Environment 71, 19–35.

Bekele, T., Cino, B.J., Ehlert, P.A.I., Van Der Maas, A.A., Van Diest, A., 1983. An evaluation of plant-borne factors promoting the solubilization of alkaline rock phosphates. Plant and Soil 75, 361–378.

Braum, S.M., Helmke, P.A., 1995. White lupin utilizes soil phosphorus that is unavailable to soybean. Plant and Soil 176, 95–100.

Buerkert, A., Bationo, A., Piepho, H.-P., 2001. Efficient phosphorus application strategies for increased crop production in sub-Saharan West Africa. Field Crops Research 72, 1–15.

Carsky, R.J., Tarawali, S.A., Becker, M., Chikoye, D., Tian, G., Sanginga, N., 1998. Mucuna-herbaceous legume with potential for multiple uses. Resource and Management Research, Monograph No. 25. IITA, Ibadan, 52pp

Cobo, J.G., Barrios, E., Kass, D.C.L., Thomas, R.J., 2002. Decomposition and nutrient release by green manures in a tropical hillside agroecosystem. Plant and Soil 240, 331–342.

Dalal, R.C., Hallsworth, E.G., 1977. Measurement of isotopically exchangeable soil phosphorus and interrelationships among parameters of quantity, intensity and capacity factors. Soil Science Society of America Journal 41, 81–86.

FAO, 1991. World Soil Resources: an explanatory note on the FAO World Soil Resources Map at 1:25 000 000 Scale. Food and Agriculture Organization of the United Nations, Rome, 58pp.

Fardeau, J.C., 1993. Le phosphore assimilable des sols: sa représentation par un modèle functionnelà plusieurs compartiments. Agronomie 13, 317–331.

Frossard, E., Fardeau, J.C., Brossard, M., Morel, J.L., 1994. Soil isotopically exchangeable phosphorus: a comparison between E- and L-values. soil Science Society of America Journal 58, 846–851.

Fyson, A., Oaks, A., 1990. Growth promotion of maize by legume soils. Plant and Soil 122, 259–266.

Gahoonia, T.S., Nielsen, N.E., 2004. Root traits as tools for creating phosphorus efficient crop varieties. Plant and Soil 260, 47–57.

Gahoonia, T.S., Nielsen, N.E., Lyshede, O.B., 1999. Phosphorus (P) acquisition of cereal cultivars in the field at three levels of P fertilization. Plant and Soil 211, 269–281.

Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237, 173–195.

Hinsinger, P., Gilkes, R.J., 1997. Dissolution of phosphate rock in the rhizosphere of five plant species grown in an acid, P-fixing mineral substrate. Geoderma 75, 231–249.

Hoffland, E., 1992. Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant and Soil 140, 279–289.

Holford, I.C.R., Mattingly, G.E.G., 1976. Phosphate adsorption and availability plant of phosphate. Plant and Soil 44, 377–389.

Horst, W.J., Kamh, M., Jibrin, J.M., Chude, V.O., 2001. Agronomic measures for increasing P availability to crops. Plant and Soil 237, 211–223.

Houngnandan, P., Sanginga, N., Woomer, P., Vanlauwe, B., van Cleemput, O., 2000. Response of *Mucuna pruriens* to symbiotic N

fixation by rhizobia following inoculation in farmers' fields in the derived Savannah of Benin. Biology and Fertility of Soils 30, 558–565.

Ibewiro, B., Sanginga, N., Vanlauwe, B., Merckx, R., 2000. Nitrogen contributions from decomposing cover crop residues to maize in a tropical derived savanna. Nutrient Cycling in Agroecosystems 57, 131–140.

IITA, 1982. Automated and semi-automated methods for soil and plant analysis. Manual Series no. 7. IITA, Ibadan, 33pp.

Jansa, J., Mozafar, A., Frossard, E., 2005. Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. Plant and Soil 276, 163–176.

Jones, D.L., Darrah, P.R., 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant and Soil 166, 247–257.

Kamh, M., Abdou, M., Chude, V., Wiesler, F., Horst, W.J., 1999a. Mobilization of phosphorus contributes to positive rotational effects of leguminous crops on maize grown on soils from Northern Nigeria. Journal of Plant Nutrition and Soil Science 165, 566–572.

Kamh, M., Horst, W.J., Amer, F., Mostafa, H., Maier, P., 1999b. Mobilization of soil and fertilizer phosphate by cover crops. Plant and Soil 211, 19–27.

Maertens, E., Thijs, A., Smolders, E., Degryse, F., Cong, P.T., Merckx, R., 2004. An anion resin membrane technique to overcome detection limits of isotopically exchanged P in P-sorbing soils. European Journal of Soil Science 55, 63–69.

Munkvold, L., Kjøller, R., Vestberg, M., Rosendahl, S., Jakobsen, I., 2004. High diversity within species of arbuscular mycorrhizal fungi. New Phytologist 164, 357–364.

Murphy, J., Riley, H.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Novozamsky, I., Houba, V.J.G., Van Eck, R., Van Vark, W., 1983. A novel digestion technique for multi-element plant analysis. Communications in Soil Science and Plant Analysis 14, 239–248.

Oladeji, O.O., Kolawole, G.O., Adeoye, G.O., Tian, G., 2006. Effects of plant residue quality, application rate, and placement method on phosphorus availability from Sokoto rock phosphate. Nutrient Cycling in Agroecosystems 76, 1–10.

Pypers, P., Delrue, J., Diels, J., Smolders, E., Merckx, R., 2006a. Phosphorus intensity determines short-term P uptake by pigeon pea (*Cajanus cajan* L.) grown in soils with differing P buffering capacity. Plant and Soil 284, 217–227.

Pypers, P., Van Loon, L., Diels, J., Abaidoo, R., Smolders, E., Merckx, R., 2006b. Plant-available P for maize and cowpea in P-deficient soils from the Nigerian Northern Guinea savanna—Comparison of E- and L-values. Plant and Soil 283, 257–270.

Russell, R.S., Rickson, J.B., Adams, S.N., 1954. Isotope equilibria between phosphates in soil and their significance in the assessment of fertility by tracer methods. Journal of Soil Science 5, 85–105.

Sanchez, P.A., Shephard, K.D., Soule, M.J., Place, F.M., Buresh, R.J., Izac, A.-M.N., Mokwunye, A.U., Kwesiga, F.R., Nditiru, C.G., Woomer, P.L., 1997. Soil fertility replenishment in Africa: an investment in natural resource capital. In: Buresh, R.J., et al. (Eds.), Replenishing Soil Fertility in Africa. SSSA Special Publ. 51. SSSA and ASA, Madison, WI, pp. 1–46.

Sanginga, N., Ibewiro, B., Houngnandan, P., Vanlauwe, B., Okogun, J.A., Akobundu, I.O., Versteeg, M., 1996. Evaluation of symbiotic properties and nitrogen contribution of *Mucuna* to maize grown in the derived savanna of West Africa. Plant and Soil 179, 119–129.

Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S., Singh, B.B., Chikoye, D., Keatinge, D., Ortiz, R., 2003. Sustainable resource management coupled to resilient germplasm to provide new intensive cereal-grain-legume-livestock systems in the dry savanna. Agriculture, Ecosystems and Environment 100, 305–314.

SAS Institute Inc, 1999. SAS User's Guide: Statistics. SAS Institute, Inc, Cary, NC.

Sharma, S.N., Prasad, R., 2003. Yield and P uptake by rice and wheat grown in a sequence as influenced by phosphate fertilization with diammonium phosphate and Mussoorie rock phosphate with or without crop residues and phosphate solubilizing bacteria. Journal of Agricultural Science 141, 359–369.

Tarawali, G., Manyong, V.M., Carsky, R.J., Vissoh, P.V., Osei-Bonsu, P., Galiba, M., 1999. Adoption of improved fallows in West Africa: lessons from mucuna and stylo case studies. Agroforestry Systems 47, 93–122.

Tian, G., Kolawole, G.O., 2004. Comparison of various plant residues as phosphate rock amendment on savanna soils of West Africa. Journal of Plant Nutrition 27, 571–583.

Van Veldhoven, P.P., Mannaerts, G.P., 1987. Inorganic and organic phosphate measurements in the nanomolar range. Analytical Biochemistry 161, 45–48.

Vanlauwe, B., Nwoke, O.C., Diels, J., Sanginga, N., Carsky, R.J., Deckers, J., Merckx, R., 2000a. Utilization of rock phosphate on a representative toposequence in the Northern Guinea savanna zone of Nigeria: response by *Mucuna pruriens*, *Lablab purpureus* and maize. Soil Biology & Biochemistry 32, 2063–2077.

Vanlauwe, B., Diels, J., Sanginga, N., Carsky, R.J., Deckers, J., Merckx, R., 2000b. Utilization of rock phosphate by crops on a representative toposequence in the Northern Guinea savanna zone of Nigeria: response by maize to previous herbaceous legume cropping and rock phosphate treatments. Soil Biology & Biochemistry 32, 2079–2090.

Vanlauwe, B., Diels, J., Lyasse, O., Aihou, K., Iwuafor, E.N.O., Sanginga, N., Merckx, R., Deckers, J., 2002. Fertility status of soils of the derived savanna and northern guinea savanna and response to major plant nutrients, as influenced by soil type and land use management. Nutrient Cycling in Agroecosystems 62, 139–150.

Vargas-Ayala, R., Rodriguez-Kabana, R., Morgan-Jones, G., McInroy, J.A., Kloepper, J.W., 2000. Shifts in soil microflora induced by velvet bean (*Mucuna deeringiana*) in cropping systems to control root-knot nematodes. Biological Control 17, 11–22.

Waigwa, M.W., Othienco, C.O., Okalebo, J.R., 2003. Phosphorus availability as affected by the application of phosphate rock combined with organic materials to acid soils in Western Kenya. Experimental Agriculture 39, 395–407.

Whitelaw, M.A., 2000. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Advances in Agronomy 69, 99–151.